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TECHNICAL MANUSCRIPT 417

SHIPPING CONTAINERS FOR ONE GALLON OR LESS OF ETIOLOGIC AGENT TESTED IN CRASH OF C-119 AIRCRAFT

Manuel S. Barbeito
Charles A. Glick

DECEMBER 1967

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

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Charles A. Glick

Agent Control Division
INDUSTRIAL HEALTH AND SAFETY OFFICE

Project 1B622401A072

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FOREWORD

Shipment of etiologic agents with adequate assurance of community safety is a technical problem being faced increasingly by medical or military laboratories and pharmaceutical manufacturers. Because Fort Detrick has for many years pioneered in the field of microbiological safety, it has undertaken the investigation of shipping containers reported herein. Although transport by land is often feasible, shipping by air is usually quicker, cheaper, and technically easier. Containers of etiologic agents shipped by air should not leak under conditions of a greater shocking impact than those shipped by land, because of the possibility of aircraft crash. Therefore, during the past 15 years, various efforts have been made to devise suitable containers that also would meet the requirements of federal regulatory agencies.

This paper describes two sizes and types of containers developed to meet technical and regulatory requirements.

The assistance of Mr. Robert D. Boyer and Mr. Edward L. Trey throughout this investigation is appreciated.

ABSTRACT

Four prototype shipping containers for 1 gallon or less of etiologic agent were assembled in compliance with Title 42, Code of Federal Regulations. Three of the containers each consisted of a No. 3 (51.7 ounces or 1,529 ml capacity) crimp-seal metal can wrapped in cotton, inserted into a No. 12 crimp-seal metal can (138.3 ounces or 4,109.5 ml capacity), and then placed in a fiberboard container with cotton forced into the space between the metal can and fiberboard container. About 0.25 inch of space was between the outer metal can and the fiberboard container. The fourth container was a 1-gallon polyethylene bottle; its sides were wrapped in cellulosic cushioning material and then it was inserted into a metal drum cushioned with vermiculite on the bottom; vermiculite was then poured on top of the bottle. This steel drum was wrapped in more cellulosic cushioning material and placed into a larger metal drum. The top of each drum was sealed with an adjustable bolt ring. Appropriate amounts of a liquid suspension of *Escherichia coli* B were placed as a test microorganism in each innermost container.

The four prototype containers were secured in a C-119 aircraft along with a principal experimental container. The plane was crashed at about 120 knots by running it up and over a 20-degree embankment 40 feet high onto desert soil. The four containers survived the crash without leaking.

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I. INTRODUCTION

Shipment of etiologic agents by air is essential to many laboratory research projects. Except for diagnostic specimens and licensed products, the packaging requirements for amounts of less than 1 gallon in the innermost container are outlined in the Code of Federal Regulations (CFR) Title 42, section 72.25 (U.S. Public Health Service), which states: "The materials shall be placed in a watertight and airtight container which shall then be enclosed in a second durable watertight and airtight container. In the case of liquid (including frozen materials), the intervening space between the containers shall be provided with sufficient absorbent material so placed as to absorb the entire contents in case of breakage or leakage. Each such double container shall then be enclosed in an individual shipping container constructed of corrugated cardboard, fiber glass, wood...." The intervening space between the two containers "shall be provided with sufficient absorbent material so placed as to absorb the entire contents in case of breakage or leakage." No test is specified for container strength, only a statement that the package be "capable of withstanding without rupture or leakage of contents, all shocks, pressure changes, or other conditions ordinarily incident to transportation handling." As far as the U.S. Public Health Service (USPHS) is concerned, such a package is acceptable for any mode of transport, but this does not mean that any commercial shipper must transport the package if it is consigned for shipment.

Containers for some hazardous materials are acceptable to other regulatory authorities only after they have passed a variety of rough handling tests, which vary greatly with the nature and volume of the material and the regulatory agency. One test that is much used is a free drop to a hard or essentially unyielding surface. As a general rule, the Air Force uses a 10-foot drop, but the Army and Navy prefer a 40-foot drop because of accidents that may occur during transfer of cargo at sea. Various tests for munition containers are described in Mil-Std-810A and 302, Mil-R-22713 (Wep) of 14 November 1960, and DA Technical Bulletin 55-100 dated 17 April 1964. For instance, The Atomic Energy Commission, in CFR Title 10, part 71, requires a free fall of 30 feet to a flat surface and a puncture drop of 40 inches. The Interstate Commerce Commission (ICC), as given in Agent T.C. George's Tariff No.19, requires for various different containers a 2-, 4-, 5-, or 6-foot drop. ICC regulations apply to commercial transport by land or water but have been adopted as minimal requirements by the Armed Services.

The impetus for a rigorous test for containers of etiologic agents arose in 1960 during an Army Chemical Corps briefing at Wright-Patterson Air Force Base, Dayton, Ohio. Sixteen federal agencies or services and four commercial airlines were represented to consider the use of Army contractor commercial airlines for unescorted shipment of containers with less than 1 gallon of etiologic agent. During the discussion it became apparent that the most convincing evidence would be a test in which an

accident was simulated by dropping the packages from a height exceeding 1,000 feet. Therefore, packages designed to meet the USPHS requirements were dropped from a 2,000- and 4,000-foot altitude, subjected to decompression tests, and dropped from 30 feet onto concrete. The packages survived without leakage and thereafter were accepted by the commercial airlines and all regulatory agencies. Any number of such packages is now acceptable in one aircraft for shipment by commercial airlines under the Official Air Transport Restricted Articles Tariff No. 6-D. Technical escort is not required. However, current regulations of the Armed Services require technical escort for any shipment in which the total volume of etiologic agent in all packages in one conveyance exceeds 3 gallons.

About this same time other container assemblies for etiologic agents were undergoing various rough handling tests under the direction of Weapons Division, Directorate of Biological Engineering, Edgewood Arsenal.* For purposes of the present discussion, the most significant test was a 1,500-foot drop onto concrete of an assembly consisting of three liquid-filled glass pint bottles stacked vertically inside individual No. 3 (51.7 ounces or 1,529 ml) tin cans in a M18A1 metal propellant container (778 cubic inches or 3.37 gal; approximately 8.5 inches maximum diameter x 26.25 inches long). The theoretical velocity attained was 3.2 feet per second (213 mph). The two containers tested impacted on their sides, opposite longitudinal seams. There was no leakage through the No. 3 tin cans although two of the six each suffered one small seam rupture. Small leaks from the glass bottles were confined to the container interiors and were absorbed by the dual-purpose cushioning.

The limitation of 1 gallon per container imposed by 42 CFR 72.25 (b) (2) (i) proved to be such an obstacle to the research program that in 1961 and 1964 amendments were inserted to permit the Surgeon General of the USPHS to approve variations in packaging and volumes if protection from leakage was at least equivalent to preceding standards. Subsequently, a container development program for air transport was undertaken by the Commodity Development and Engineering Laboratory of Fort Detrick and by the Department of the Air Force, Air Force Armament Laboratory, Eglin Air Force Base, Florida. Test criteria were established for the shipping container in an AFRDQR, with subsequent minor modifications. One test of major importance to the USPHS is impaction of an aircraft, with the container inside, perpendicularly into an infinite fixed mass at a terminal velocity of 1 knots (150 mph).

Opportunity to determine the effect of crash of an aircraft upon containers holding less than 1 gallon was provided during a modified experimental crash.

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* This group is now part of Production and Maintenance Division, Commodity Development and Engineering Laboratory, Fort Detrick.

A C-119 aircraft was crashed by causing it to run up a 20-degree embankment over a 40-foot concrete wall at approximately 120 knots (138 mph). In addition to the principal experimental container under test, four small shipping containers were placed in the aircraft to determine whether they would remain intact and biologically tight.

Three 404-700 (No. 3) tin cans each containing 1,400 ml of Escherichia coli culture were packaged in accordance with CFR Title 42, section 72.25 USPHS, and a 1-gallon plastic container was packaged as a developmental prototype under the PEMA* program administered by Production and Maintenance Division, Fort Detrick. E. coli strain British 162, count 2 x 10^{10} organisms per ml, was used as the test agent in these four containers so as not to interfere in any manner with the test simulant Serratia marcescens contained in the principal experimental container. Prior to final packaging, all exterior surfaces of the inner cans and outside containers were decontaminated with 5% tricresylic acid (Lysol) to remove residual E. coli. Subsequent surface sampling showed the containers to be free of E. coli.

An additional indication that it is practical to design packages for a 1,000- to 1,500-foot drop test is found in experiments by the USPHS. Diagnostic specimens packaged according to 42 CFR 72.25 survived this drop onto hard sunpacked ground without leakage.**

II. CONTAINER PREPARATION

A. NO. 3 CANS

Each of three No. 3 tin cans (can size 404-700, 4.25 inches diameter x 7 inches high), capacity 51.7 oz, at 68 F contained 1,400 ml of E. coli liquid suspension. Each was crimp-sealed, wrapped with absorbent cotton (approximately 8 oz), and then placed in a No. 12 1-gallon crimp-sealed tin can (can size 603-812). This assembly was placed in a spirally wound telescopic fiber container (metal top and bottom, cardboard asphalt impregnated sides), Military Specification MIL C-3955A, 16 February 1960. Outer dimensions were 7 1/8 inches diameter by 9 5/16 inches high. Absorbent cotton then was forced into the void (approximately 0.25 inch) between the tin can and fiber container. The fiber container was sealed with 1-inch wide tape at the top-to-bottom joint (Fig. 1 through 3). This fiber container is designated under 42 CFR as the "individual shipping container."

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* Procurement of Equipment and Missiles, Army.

** Kokko, U.P.; Stuart, J.; Taylor, G. 1960. Mailing of infectious specimens for diagnostic purposes. Public Health Rep. 75:979-984.

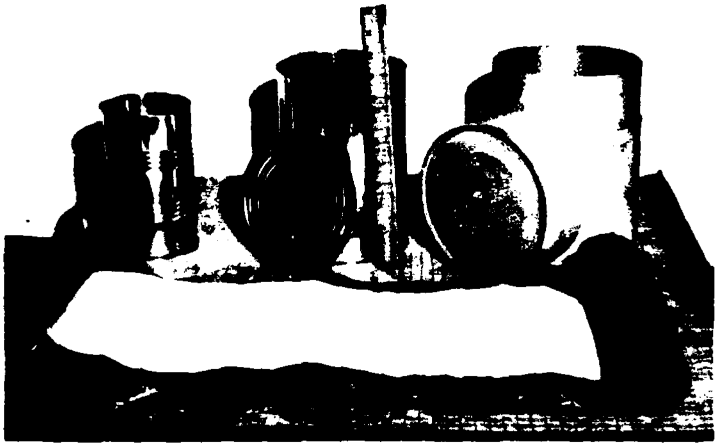

Figure 1. Components of Packages A, B, and C. Left, No. 3 can; center, No. 12 can; right, spirally wound fiber can; front, absorbent cotton.

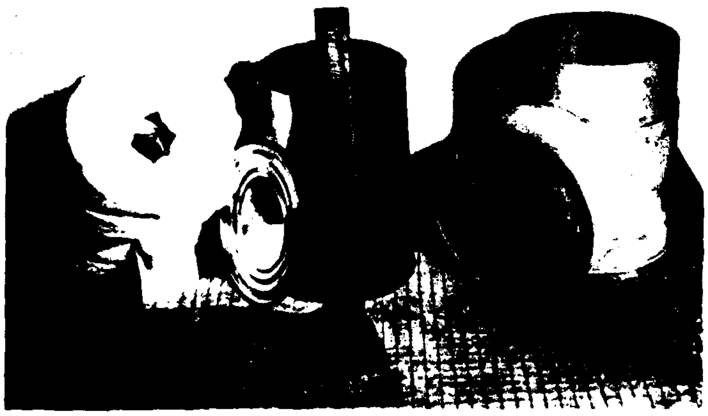

Figure 2. Components of Packages A, B, and C, Partially Assembled.

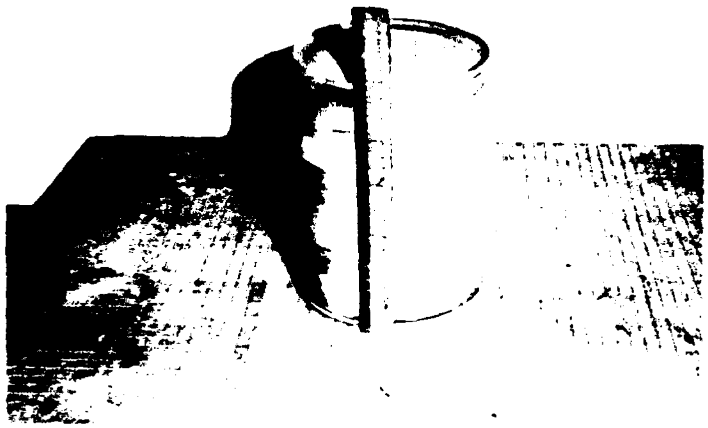

Figure 3. Telescopic, Asphalt Impregnated, Spiral Wound Fiber Individual Shipping Container Used in Packages A, B, and C.

B. PEMA CONTAINER

The PEMA prototype container consisted of a 1-gallon polyethylene plastic bottle with polypropylene cap and two heavy steel cylindrical drums. After filling the bottle with 4,200 ml liquid suspension of E. coli. the cap was secured to the bottle by tape (Military Specification No. MIL-T-43036).

The bottle was wrapped with 14 ounces of longitudinally compressed cellulosic cushioning material, Type I - Water Absorbent, Class A - Low Tensile (Federal Specification PPP-C-843a, 1 December 1960 and amended changes) (See Appendix A). The wrapped bottle was placed with cardboard spacers inside the first steel drum. The bottom and top spaces around the bottle were filled with 3 pounds, 6 ounces of expanded vermiculite, size IV (Military Specification MIL-V-21628, 14 October 1958). This first steel drum was wrapped in 3 pounds, 15 ounces of longitudinally compressed cellulosic cushioning material, top, bottom, and sides, and placed in the steel drum overpack with its cardboard and wooden filler blocks (Fig. 4 and 5).

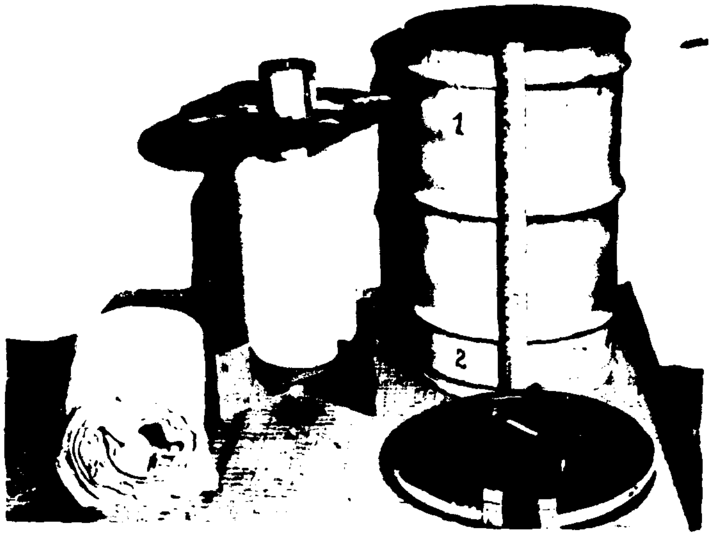

Figure 4. Components of Package D. Left, cellulosic material; center, 1-gallon plastic bottle; right, inner steel drum.

Figure 5. Components of Package D. Left, outside steel drum; center, 1-gallon plastic bottle; right, inner steel drum.

C. SECURING OF CONTAINERS IN AIRCRAFT

The four containers were located in the cargo area of the aircraft adjacent to each corner of the principal experimental container (Fig. 6). One of the 1,400-ml containers (B package) was placed in ice inside a 31-inch long, 14-inch wide, 16-inch high insulated aluminum chest (normal manner for shipment of agent in this size container). All four containers were located as shown in Figure 6 and secured as shown in Figures 7 through 10.

III. RESULTS AFTER CRASH OF AIRCRAFT

A. LOCATION AND CONDITION OF CONTAINERS AFTER CRASH

Following the crash of the C-119, all four containers were thrown clear of the wreckage at a distance from 2 to approximately 200 feet. Package A was beneath the wings. Its metal bottom was open, but otherwise the package was intact. The aluminum ice chest (package B) was thrown out of the aircraft immediately forward of the fuselage and crushed. However, the individual shipping container (fiberboard) had been ejected from the ice chest and lay approximately 1 foot away with its metal bottom that was torn loose. Otherwise this container was intact. The individual shipping container of package C was separated from its double tin can assembly and lay approximately 20 feet away. The metal top was torn from the fiber sides and was about 7 feet from the other part. The nested metal cans were intact except for a tear (approximately 1 inch) in the No. 12 can. The PEMA container remained secured to the cylindrical camera box with its sides crushed from the force exerted on its tiedown chain. The outer steel drum and the inner steel drum were wedged together, but neither was ruptured. This container was found on top of the embankment approximately 200 feet from the aircraft impact area. Damage to the four shipping containers is shown in Figures 11 through 14.

B. SURFACE SAMPLING FOR RECOVERY OF *E. COLI*

Two types of surface sampling were used. The Swube* cotton-tipped applicator was prepared for use by adding 2 cc of a sterile solution consisting of 0.2% gelatin and 0.4% anhydrous dibasic sodium phosphate to each tube. Immediately before use the cotton was moistened with the solution in the tube by pushing the applicator to the bottom of the tube. The 2 cc of moistening solution were discarded as the sample was taken. The moistened swab sampled 3.5 square inches of surface (e.g., 7 x 0.5 inches), which is roughly equivalent to the surface area of the Rodac** plate. After sampling, the cotton swabs were rolled across eosin methylene blue plates and the plates were incubated. The plates were

* Swube, Catalog No. 2009, with 17 x 100 mm test tube, Falcon Plastics, 5500 W. 33rd St., Los Angeles, Calif. 90045.

** Rodac plate, Catalog No. 1034, Falcon Plastics.

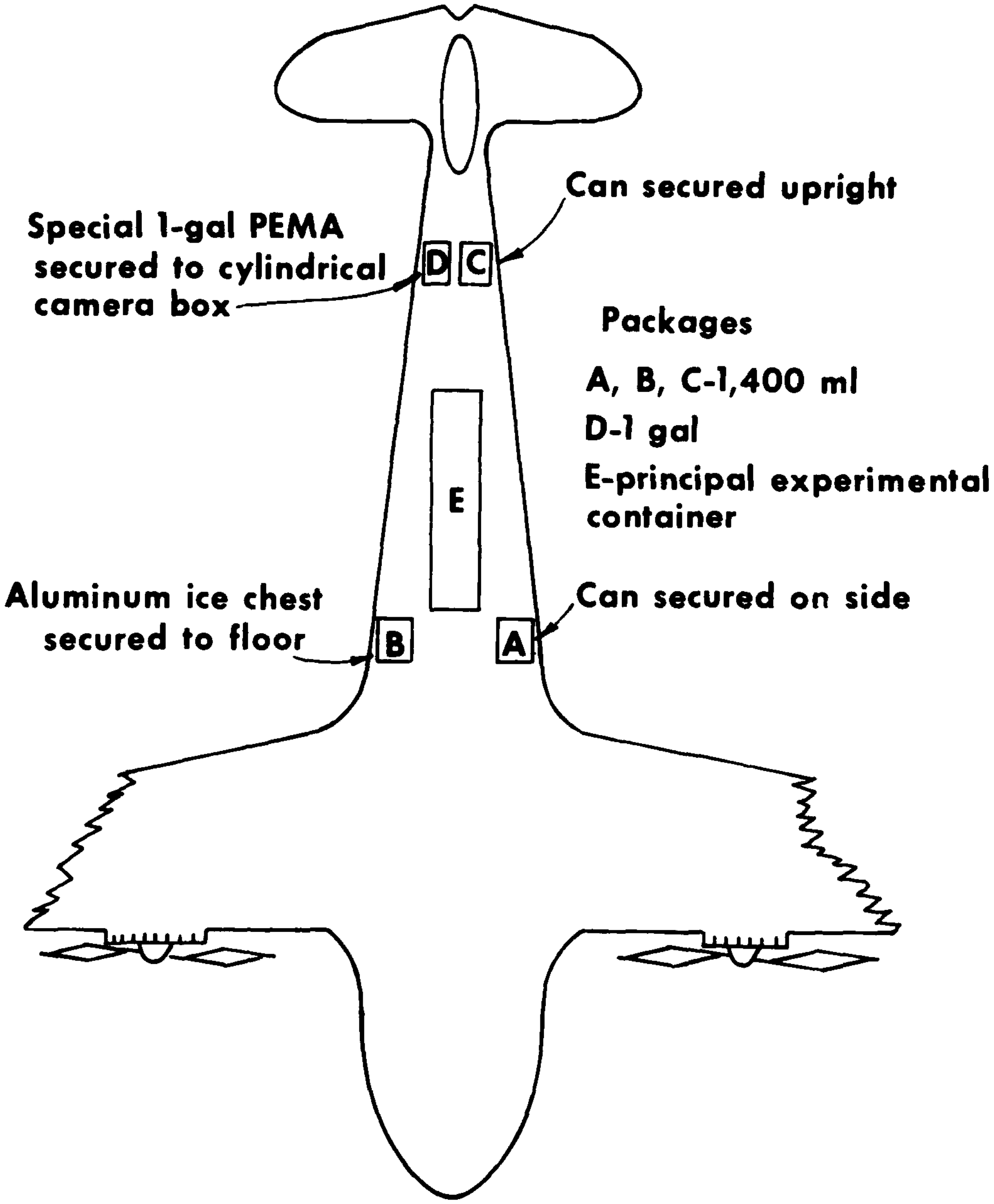

Figure 6. Location of Packages in Aircraft.

Figure 7. Package A Secured in Aircraft. 1,400-ml container in horizontal position.

Figure 8. Package B Secured in Aircraft. 1,400-ml container in ice inside aluminum ice chest.

Figure 9. Package C Secured in Aircraft. 1,400-ml container in vertical position.

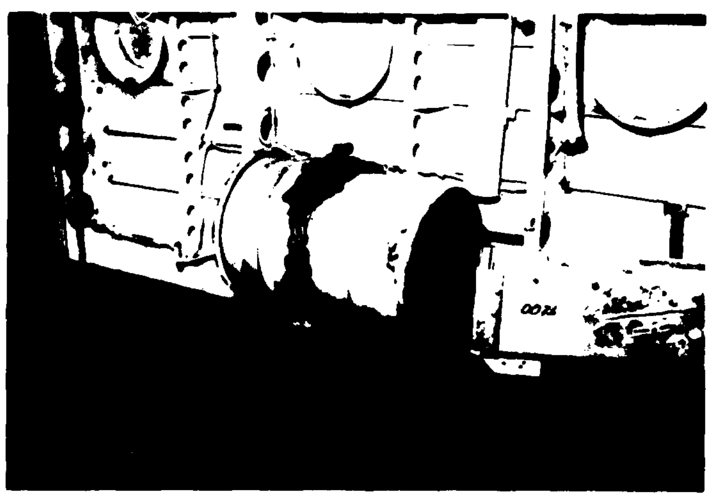

Figure 10. Package D Secured in Aircraft. PEMA, 1-gallon container.

Figure 11. Package A after the Crash.
The No. 12 can was removed by hand from the fiber container and then opened to show the condition of the No. 3 can (no leakage).

Figure 12. Package B after the Crash.
The package has been disassembled to show that the metal ottom of the fiberboard container was torn loose and that the No. 12 and No. 3 cans were dented but intact (no leakage). Ice chest was crushed.

Figure 13. Package C after the Crash.
The No. 12 can was removed by hand from the fiber container and then opened to show the condition of the No. 3 can (no leakage).

Figure 14. Package D (the PEMA Container) after the Crash.
Disassembled: left, vermiculite; center, nested steel drums; right, 1-gallon plastic bottle (no leakage).

examined at 24 and 48 hours for the typical black sheen of E. coli colonies. The second sampling method used Rodac plates.* The Rodac plates were prepared by adding 16.5 cc of sterile eosin methylene blue agar to each plate so that the agar had a convex surface mounded up above the rim of the plate. Samples were taken by removing the lid and pressing the exposed agar surface onto the surface to be sampled. The Rodac plates were incubated for 48 hours. All plates were examined at 24 and 48 hours for E. coli colonies. Incubation temperature was 37 C for E. coli.

The sampling method for recovery of E. coli from the four shipping containers was as follows: (i) for the PEMA outside steel drum, six Swube samples and six Rodac plates; (ii) for all other inner and outer containers, three Swube samples and three Rodac plates each.

Before any of the containers were removed from the crash site, surface samples for E. coli were taken from the individual shipping containers and also from the No. 12 tin can in package C. Subsequently the containers were collected and taken to a central work station. After opening all containers except the innermost one, additional surface samples were taken from each of the two inner containers.

All surface samples were negative for E. coli. The results showed that the three No. 3 cans and the PEMA container survived without leakage or appreciable damage in the aircraft crash at approximately 120 knots.

IV. DISCUSSION

Sanitary crimp-seal metal cans have been used successfully for a number of years without obvious leakage upon arrival at distant destinations. During the process of crimping cans, inspection must be frequent to insure that the crimp is intact and complete, because canner mechanisms have a tendency to slip and cause misalignment of crimping wheels.

AMC Regulation 385-101 at present requires granular calcium hypochlorite to be interspersed in absorbent cotton between the first and second inner containers. This requirement was based upon the desire for a bactericidal action on a liquid etiologic agent in the event of leakage. However, use of calcium hypochlorite in the above manner has created a potential explosive mixture. After a container of etiologic agent is opened inside a biological safety cabinet it is necessary to autoclave the discarded materials. Autoclaving cotton and calcium hypochlorite in the presence of small amounts of organic materials such as oil, dibutyl phthalate, or contents from a marking pen wick can cause a violent exothermic reaction. Furthermore, unless a package containing dry calcium hypochlorite is refrigerated

*Ulrich, J.A. 1964. Technic of skin sampling for microbial contaminants. Health Lab Sci. 1:133-136.

properly, an internal pressure can develop. This pressure is caused by condensation inside the can. The moisture reacts with calcium hypochlorite to release oxygen that accounts for the internal pressure. Because of these chemical reactions, it is strongly recommended that calcium hypochlorite not be used in containers used for shipment of etiologic agents.

The PEMA container is a developmental item. Therefore, changes will be made before it becomes a standardized container for shipment of etiologic agent. These initial tests indicate that the two types of cellulosic cushioning material provided adequate shock absorbency and liquid absorbency.

Either absorbent cotton or vermiculite (size IV) acts as a suitable shock absorber, and both possess adequate liquid absorbency to meet specifications. The vermiculite is dusty and difficult to handle in a biological cabinet during packaging and opening. Absorbent cotton is operationally preferred to vermiculite at present.

For this test, the containers were fastened to the aircraft body less securely than normally. This was done so that more force would be exerted on the packages by thrust and deceleration. Under normal conditions of stowage it is probable that the packages would survive a crash with less damage than in this test.

APPENDIX A

ABSORBENCY OF PACKING MATERIAL USED ON PEMA CONTAINER

Title 42 of the Code of Federal Regulations on the shipment of etiologic agents states, "In the case of liquid (including frozen materials), the intervening space between the containers shall be provided with sufficient absorbent material so placed as to absorb the entire contents in case of breakage or leakage." The prototype 1-gallon PEMA container is governed by this regulation. Therefore, the absorbency of the packing material was determined.

A. ABSORBENCY TESTS

Two types of cellulosic cushioning material and vermiculite were obtained for testing. One cushioning material was longitudinally compressed and crimped into flat sheets consisting of several layers; the other was rolled uncrimped sheet material. One cubic inch of test materials was immersed in distilled water. The weight gain was determined at various times and per cent absorption was calculated, with results shown in Table 1.

TABLE 1. LONGITUDINALLY COMPRESSED CELLULOSIC MATERIAL

Sample No.	Dry Wt, g	Immersion Time, min	Wet Wt, g	Net Gain, g	Absorption, %
1	1.43	5	10.33	8.90	623
2	1.45	10	11.55	10.10	697
3	1.40	15	13.28	11.88	817
4	1.42	20	13.99	12.57	885
5	1.43	25	16.84	15.41	1,080
6	1.39	30	21.36	19.97	1,435

B. PACKAGING

The weight of longitudinally compressed cellulosic material Type I placed around the 1-gallon plastic bottle in the PEMA container was 14 oz. In 30 minutes this amount of material will absorb approximately 12.5 lb. (1.5 gal) of liquid (Table 1).

The weight of sheet cellulosic material Type I placed around the first steel drum in the PEMA container was 3 lb., 15 oz. In 30 minutes this material will absorb approximately 19.2 lb. (2.3 gal) of liquid (Table 2).

TABLE 2. SHEET CELLULOSIC CUSHIONING MATERIAL

Sample No.	Dry Wt, g	Immersion Time, min	Wet Wt, g	Net Gain, g	Absorption, %
1	1.97	5	6.52	4.55	231
2	1.93	10	6.38	4.45	230
3	1.91	15	5.00	4.09	214
4	2.12	20	6.33	4.21	198
5	2.27	25	6.68	4.41	194
6	1.80	30	10.5	8.7	483

The weight of vermiculite (size IV) used across the top and bottom surfaces between the first and second steel drums of the PEMA container was 3 lb., 6 oz. (1290 g). In 30 minutes, this amount of vermiculite will absorb approximately 4.6 lb. (0.54 gal) of liquid (Table 3).

TABLE 3. VERMICULITE

Sample No.	Dry Wt, g	Immersion Time, min	Wet Wt, g	Net Gain, g	Absorption, %
1	19.2	5	39.5	20.3	105
2	19.5	10	40.5	21.0	107
3	19.2	15	42.2	23.0	120
4	19.5	20	47.2	27.7	142
5	20.0	25	45.2	25.2	126
6	19.5	30	45.4	25.9	135

The conclusion from these tests is that either the crimped or the rolled Type I sheet cellulosic material is satisfactory as an absorbent when placed as described between the 1-gallon plastic bottle and the first steel drum in the PEMA container. Either will absorb the liquid contents of the plastic bottle.

Unclassified
Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author) Department of the Army Fort Detrick, Frederick, Maryland 21701	2a. REPORT SECURITY CLASSIFICATION Unclassified 2b. GROUP

3. REPORT TITLE

SHIPPING CONTAINERS FOR ONE GALLON OR LESS OF ETIOLOGIC AGENT TESTED IN CRASH OF C-119 AIRCRAFT

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)

5. AUTHOR(S) (First name, middle initial, last name)

Manuel S. Barbeito
Charles A. Glick

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
December 1967	24	2

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
b. PROJECT NO. 1B622401A072	Technical Manuscript 417
c.	9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)
d.	

10. DISTRIBUTION STATEMENT

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11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY Department of the Army Fort Detrick, Frederick, Maryland 21701

13. ABSTRACT

Four prototype shipping containers for 1 gallon or less of etiologic agent were assembled in compliance with Title 42, Code of Federal Regulations. Three of the containers each consisted of a No. 3 (51.7 ounces or 1529 ml capacity) crimp-seal metal can wrapped in cotton, inserted into a No. 12 crimp-seal metal can (138.3 ounces or 4,109.5 ml capacity), and then placed in a fiberboard container with cotton forced into the space between the metal can and fiberboard container. About 0.25 inch of space was between the outer metal can and the fiberboard container. The fourth container was a 1-gallon polyethylene bottle; its sides were wrapped in cellulosic cushioning material and then it was inserted into a metal drum cushioned with vermiculite on the bottom; vermiculite was then poured on top of the bottle. This steel drum was wrapped in more cellulosic cushioning material and placed into a larger metal drum. The top of each drum was sealed with an adjustable bolt ring. Appropriate amounts of a liquid suspension of *Escherichia coli* B were placed as a test microorganism in each innermost container.

The four prototype containers were secured in a C-119 aircraft along with a principal experimental container. The plane was crashed at about 120 knots by running it up and over a 20-degree embankment 40 feet high onto desert soil. The four containers survived the crash without leaking.

14. Key Words
*Containers
*Safety
*Packaging
*Transporting

DD FORM 1 NOV 65 1473 REPLACES DD FORM 1473, 1 JAN 64, WHICH IS OBSOLETE FOR ARMY USE.

Unclassified
Security Classification